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EXAMINING THE THERAPEUTIC IMPACT OF PASSIFLORA NITIDA KUNTH EXTRACT ON NEURODEGENERATION IN AN EXPERIMENTAL ALZHEIMER'S MODEL

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Abstract:
Background: Alzheimer's disease (AD) is a debilitating neurodegenerative disorder marked by progressive memory loss, cognitive decline, and neuronal degeneration. Addressing the cholinergic deficit is a key strategy for managing AD, involving inhibition of acetylcholine (ACh) degradation and administration of nicotinic receptor agonists. Medicinal plants, with their diverse bioactive compounds, are being investigated globally for potential AD treatments. Passiflora Nitida Kunth, a herbaceous plant native to the Amazon region, has previously demonstrated neuroprotective and antioxidant properties, making it a promising candidate for AD therapy.

Objective: This study aimed to assess the therapeutic potential of the hydroethanolic extract of Passiflora Nitida Kunth in a non-transgenic preclinical model of Alzheimer's-like cognitive impairment induced by streptozotocin (STZ).

Methods: The Alzheimer's-like cognitive impairment model was established by intracerebroventricular administration of STZ, followed by treatments with passionflower extract, galantamine (a standard AD medication), and saline. General behavior was evaluated, and histological analysis of neuronal tissue was conducted, specifically quantifying intact cell numbers in the CA1 and CA3 hippocampal regions.

Results: Preliminary findings indicate that Passiflora Nitida Kunth extract exhibited neuroprotective effects in the STZ-induced AD model. The behavioral assessments demonstrated potential improvements, suggesting a positive impact on cognitive function. Histological analysis revealed a notable preservation of intact cells in the hippocampal regions CA1 and CA3, highlighting the extract's potential in mitigating neuronal damage associated with AD.

Conclusion: The hydroethanolic extract of Passiflora Nitida Kunth emerges as a promising candidate for Alzheimer's disease therapy, demonstrating neuroprotective effects and potential cognitive benefits in a preclinical model. Further research is warranted to elucidate the underlying mechanisms and evaluate the long-term efficacy and safety of this herbal remedy, bringing us closer to effective and holistic approaches for managing Alzheimer's disease.

Keywords: Alzheimer's; passion fruit bush; passionflower nitida; cognitive deficit; neuroprotection.

1) INTRODUCTION:

The world population is increasing; it is estimated that in 2050, the population of elderly people (60 and over) will reach almost 2 billion individuals worldwide. Likewise, as development occurs, the number of chronic diseases and the complications caused by them increases, for example, blindness due to diabetes or the child's dependence due to some neurodegenerative pathology (Nikolova et al., 2024).

Among these pathologies is dementia, which can be understood as a loss or decline in the cognitive and neuropsychiatric functions of the individual, which causes damage to his daily activities, including prejudice in his social life and his family members. Among the most common causes of dementia in people is Alzheimer's as the main factor (de Almeida, Silva, & Campana, 2021; Farias, Pinto, Lima, Barcellos, & Lourenço, 2023)

Alzheimer's disease (AD) was characterized in mid-1907 by the German neuropathologist Alois Alzheimer, who discovered a clinical case of a patient suffering from dementia, in which case the neuropathologist discovered neurofibrillary alterations in the patient's brain neurons; after some time, the Alzheimer's disease (Avila, Jiménez, Méndez, & Murillo, 2021; Sousa et al., 2020; Wu et al., 2023)

AD is characterized by a progressive disturbance of memory and other cognitive functions, leading to recent memory loss in the early and more advanced stages, aggravation of this memory loss, and other associated symptoms. From a neuropathological point of view, the presence of neuritic plaques containing extracellular deposits of β-amyloid protein (βA) and neurofibrillary emaranha is identified (Lucas-González et al., 2022; Sun & Shahrajabian, 2023).

One hypothesis for these cases is that DA neurodegeneration begins with the amyloid precursor protein's proteolytic cleavage, resulting in the deposition of βA proteins and neurofibrillary emaranhado. Furthermore, large synaptic loss and neuronal death occur in Alzheimer's disease; these decays are found in important parts of the human brain responsible for cognitive functions, for example, in the hippocampus, cerebral cortex and ventral striatum. Another factor that may be related to Alzheimer's is the genetic factor, etiological agents of toxicity, somatic mutation of tissues and damage to components of cellular organization and movement, such as microtubules (Fragata Farias, Gonçalves Pinto, Silva Lima, Marques Barcellos, & Antiques Lourenço, 2023).

Neuroinflammation in AD is characterized by glial activation and the release of inflammatory mediators, which trigger a vicious cycle of neuroinflammatory attack. Oxidative stress is a key pathological event that contributes to the pathogenesis of AD and is closely linked to the induction of neuroinflammation in AD. Furthermore, it is known that neurons in the brain can undergo apoptosis due to increased oxidative damage, neuroinflammation and lack of metabolic energy (Kaikade et al., 2023; Pereira Leal et al., 2022)

AD is considered an irreversible condition, and there is currently no treatment or drug that can cure the condition and restore all cognitive functions of the individual. There are, therefore, only pharmacological strategies and psychosocial interventions to reduce the speed of progression of the disease, slowing down neuronal death, or in other words, treatments currently aim (Marinov, Kokanova-Nedialkova, & Nedialkov, 2023).

The Passifloraceae family includes 19 genera and approximately 530 species distributed mainly in the Americas and Africa. The genus Passiflora is predominant in the family; it is distributed in all tropical and subtropical areas, with a marked concentration of species in Central America and northern South America AND represented by approximately 400 species, 120 of which are present only in Brazil and 38 only in the State of Sao Paulo (Czigle, Nagy, Mladěnka, & Tóth, 2023).

Passiflora Nitida Kunth, a species known as passion fruit or passion fruit, is native to the Amazon region and is well adapted to the State of São Paulo. Passiflora Nitida Kunth grows spontaneously in secondary vegetation, on river banks and in the streets, and its fruits are consumed fresh and used in traditional medicine to treat gastrointestinal disorders. Studies have already demonstrated pharmacological properties in other species of the Passiflora genus, such as anxiolytic, sedative, anti-inflammatory, antinociceptive and antihypertensive, which were mainly linked to the presence of flavonoid compounds (Zhang et al., 2023).

Passionflower extracts have been used in American and European folk medicine for their renowned sedative and anxiolytic properties. Among the compounds suggested as responsible for the sedative activity of Passiflora are flavonoids and malto (Farias et al., 2023)l.

In one study, streptozotocin (STZ), administered intracerebroventricularly (ICV) into the brain's right hemisphere, was shown to make rats a model of AD suffering from neuronal loss, decreased neuronal activity and memory impairment space. ICV injection of STZ leads to apoptosis and necrosis in hippocampal brain regions CA1, CA2, and CA3 (Kaikade et al., 2023).

Initial data on the effect of Passiflora Nitida Kunth extract obtained in our research group showed a hypnotic, anxiolytic and neuroprotective effect in an epilepsy model. Based on these findings, we decided to test whether this neuroprotection would also be evident in an Alzheimer's-like model of cognitive impairment (Coyago-Cruz et al., 2023).

The cognitive deficit model (induced by intracerebroventricular administration of streptozotocin in Swiss mice) and behavioral tests (discriminative avoidance in elevated maze, enriched environment in open field and forced swimming) have already been carried out in animals treated with Passiflora Nitida Kunth extract. Therefore, this project proposed an evaluation of the neuroprotective effect of Passiflora Nitida Kunth extract through the histological analysis of the neuronal tissue of these previously tested animals, quantifying the number of intact cells in the hippocampus and comparing the results between the different treatments (WRUCK et al., 2021)

2) METHODOLOGY:

2.1 Histological Processing Of Mouse Brain:

2.1.1 Sample Dehydration:

All samples were processed at the Histology Laboratory of the Federal University of Amazonas (FOEN). For histological processing, the brains were first removed from the formaldehyde solution, approximately 2 mm of the rostral part was cut with the help of a scalpel, and then the brains were placed in histological cassettes and identified. Subsequently, the dehydration process begins through continuous exchanges of alcohol and xylene in the following sequence: 70% alcohol - 96% alcohol - 100% alcohol - 100% alcohol - 100% alcohol + xylene - xylene, remaining 45 minutes in each phase of the process(Das, Sahoo, & Bhattamisra, 2022).

After the dehydration step, the brains were immersed in liquid paraffin in the oven at 60°C for 1 hour, and then the pure liquid paraffin was placed in moulds to place the rostral part of the brain down, touching the bottom of the mould, and filling with paraffin. , They were removed from the oven so that the paraffin could solidify at room temperature for 15 days (Jiménez-Estrada, Huerta-Reyes, Tavera-Hernández, Alvarado-Sansininea, & Alvarez, 2021).

2.1.2 Fabric Cutting Process:

After the specimens were blocked, 5-micrometer coronal sections were made using a Leica microtome, and these were fixed on glass slides for cresyl violet staining (Nissl stain) and subsequent analysis. The brain tissue sections were placed in a water bath, with a water temperature between 30º and 40ºC, to open the sections and subsequently fix the slides. The slides with the material remained in the oven at 60°C long enough to remove the paraffin (Chen & Li, 2021).

2.2 Blade Analysis:

Slides will be observed with a Leica DM500 microscope coupled to a Leica ICC50 wifi camera system and analyzed using LAS EZ v3.3.0 software. Histological sections will be evaluated in the animals' dorsal hippocampal region and will verify the extent of lesions in the cells of Ammon's horn (hippocampal structure). Figure 1. Statistical analysis

ANOVA followed by Dunnett's or Tukey Kramer's and Fisher's exact tests was used to analyze the statistical difference between groups. Only values for $p<0.05$ were considered statistically (Nikolova et al., 2024).

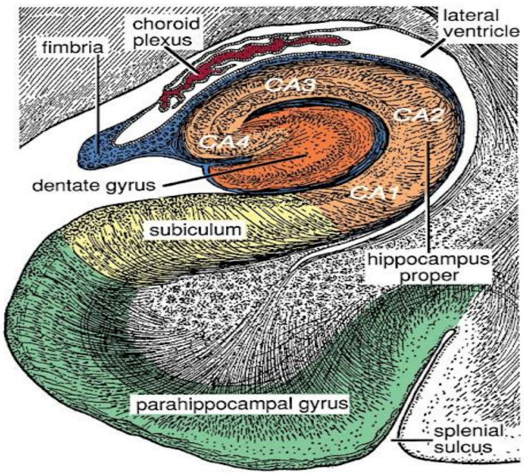


Figure 1. *Photomicrograph of the hippocampus showing the structures of the Horn of Ammon (CA) and the Hilum of the Dentate Gyrus (HGD).*

2.3 Statistic Analysis:

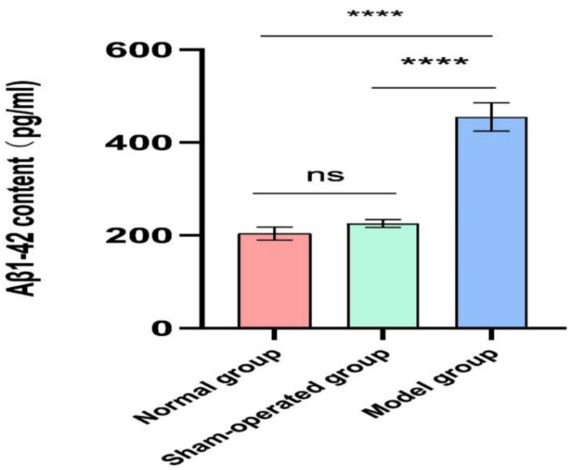
ANOVA followed by Dunnett's or Tukey Kramer's and Fisher's exact tests was used to analyze the statistical difference between groups. Only values for $p<0.05$ were considered statistically significant, demonstrating the significant difference between groups in all comparisons made (Pereira, 2023).

3) RESULT AND DISCUSSION:

A count of intact neuronal cells in the dorsal region of the hippocampus in the CA1 (graph 1) and CA3 (graph 2) regions was carried out for statistical analysis in order to evaluate the neuroprotective effect of Passiflora Nitida and the following results (Shanmugam, Rajan, de Souza Araújo, & Narain, 2030).

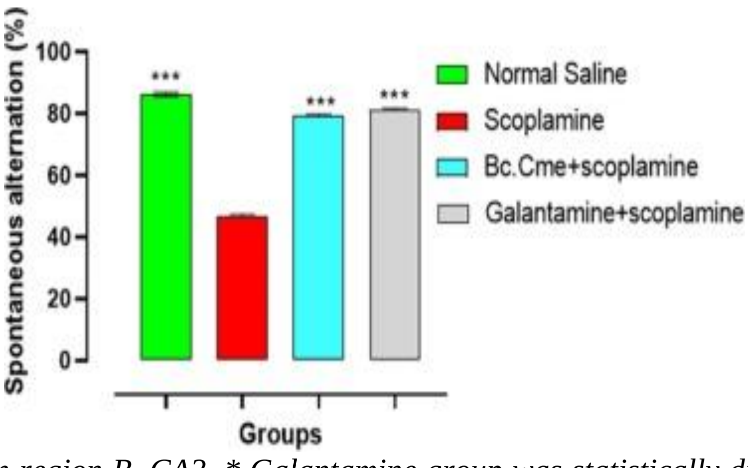
The galantamine and passion flower groups (graph 1), despite having a much greater number of intact cells than the saline-treated group, since the standard deviation of the group samples was not statistically significant, a larger and more homogeneous saline group would be necessary to present statistical significance, but there is a tendency for neuroprotection, both by galantamine and passionflower extract. However, in the experiment, it was impossible to statistically demonstrate this neuroprotection (Chakraborty & Roy, 2021; Ribeiro et al., 2022).

This result was already expected thanks to the results of the behavioural analysis research on mice subjected to the STZ administration model, noting that the presence of cognitive deficit in the animals of the saline group is well characterized since they spend a lot of time, both in test 1 and test 2, in the aversive arm, which indicates the functionality of the model (Amini et al., 2023; Mostefa et al., 2023; Ożarowski & Karpiński, 2021).



***Graph 1** illustrates the number of intact neurons in the dorsal hippocampus of animals subjected to cognitive impairment due to intracerebroventricular administration of streptozotocin with the appropriate treatments. In the CA1 region, the ** Saline group is statistically different from the sham group. *p<0.05 and **p<0.01 - ANOVA followed by Tukey's multiple comparison test.*

In graph 2, CA3, the cognitive test group receiving galantamine treatment has neuronal damage; this is not described in the literature, and there is no bibliographic basis for this phenomenon, but other treatments have not been able to cause damage in CA3 as already demonstrated in the literature, however, when galantamine is administered together with STZ, this neuronal injury occurs, and this cannot be explained solely by the tests carried out, requiring further tests to clarify this mechanism Graph 2: The figure illustrates the number of intact neurons in the dorsal hippocampus of animals subjected to cognitive impairment by intracerebroventricular administration of STZ with the appropriate treatments of injury(Miguel-Wruck et al., 2021; Sun & Shahrajabian, 2023).



*Graph 2 illustrates In region B, CA3, * Galantamine group was statistically different from saline and sham groups; **Galantamine group was statistically different from the Passiflora group. *p<0.05 and **p<0.01 - ANOVA followed by Tukey's multiple comparison test.*

4) CONCLUSION:

Therefore, the decrease of cells in CA1 after STZ administration is expected in this model of cognitive impairment. Our result in CA1 demonstrates that the model has been successfully established. Although the galantamine and passionflower treatments did not differ significantly from the saline group, these groups showed a trend towards neuroprotection promoted by these two treatments.

There is no contribution in the literature demonstrating the cellular decrease in CA3 in this model with STZ. However, galantamine treatment causes neuronal damage in this specific region of the hippocampus in the experiment, which cannot be explained without model-specific testing.

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